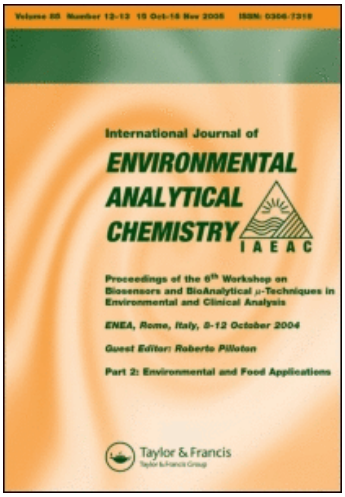

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Organophosphorus Compounds in Mediterranean Coastal Waters and Biota Samples Using Gas Chromatography with Nitrogen-Phosphorus and Chemical Ionization Mass Spectrometric Detection

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To cite this Article Barceló, Damià , Porte, Cinta , Cid, Jose and Albaigés, Joan(1990) 'Determination of Organophosphorus Compounds in Mediterranean Coastal Waters and Biota Samples Using Gas Chromatography with Nitrogen-Phosphorus and Chemical Ionization Mass Spectrometric Detection', International Journal of Environmental Analytical Chemistry, 38: 2, 199 — 209
To link to this Article: DOI: 10.1080/03067319008026927
URL: http://dx.doi.org/10.1080/03067319008026927

Intern. J. Environ. Anal. Chem., Vol. 38, pp. 199–209
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DETERMINATION OF ORGANOPHOSPHORUS COMPOUNDS IN MEDITERRANEAN COASTAL WATERS AND BIOTA SAMPLES USING GAS CHROMATOGRAPHY WITH NITROGEN-PHOSPHORUS AND CHEMICAL IONIZATION MASS SPECTROMETRIC DETECTION

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An analytical method is described for the determination of organophosphorus compounds in water and biota samples. This includes solvent extraction, a clean up procedure using silica gel and gel permeation chromatography with nitrogen-phosphorus and positive and negative chemical ionization mass spectrometric detection.

Organophosphorus compounds were measured in river waters and biota samples (*Mytilus galloprovincialis*, *Mullus barbatus* and *Gambusia affinis*) from the Spanish Mediterranean coast. The results show that phosphates are only found in water samples affected by industrial activities, whereas organophosphorus pesticides, exhibiting higher bioconcentration factors, are found in biota samples.

KEY WORDS: Organophosphorus compounds, chemical ionization mass spectrometry, gas chromatography with nitrogen-phosphorus detection, gel permeation chromatography, analysis of water and biota samples.

INTRODUCTION

Organophosphorus (OP) compounds consist of a group of roughly 250 chemicals manufactured all over the world. Approx. 140 of these compounds are pesticides and the remaining are mainly industrial chemicals used as flame retardants, plasticizers and industrial hydraulic fluids and solvents.

Organophosphorus pesticides are currently used in agriculture or animal husbandry for crop protection and/or elimination of ectoparasites.[1] The available figures on the production of OP pesticides are scarce. A worldwide consumption of 24,000 tons of malathion during the years 1980/81 has been estimated. The consumption of OP pesticides in the Mediterranean countries during the period 1981–83 was 40,000 tons, Italy being the main consumer.[2] Non-pesticide OP's have been widely used since the 1940's in industrial and domestic products such as fire retardant plasticizers and high temperature functional fluids. The use of fire retardants during the 1960's and 1970's increased greatly due to the demand for improved fire safety in commercial products made from synthetic polymers.[3,4]

The OP pesticides usually exhibit $\log K_{ow}$ values of between 3–4, although

the range varies from −2.56 for dimefox to 5.95 for temephos.[5] For the non-pesticides, the lowest log K_{ow} value, 1.47 is for tris(2-chloroethyl)phosphate, and the highest, 6.08, for cumylphenyl diphenyl phosphate.[4]

Because of the extensive use of OP pesticides, the risk of contamination affects different types of aquatic environments, namely rivers, estuaries, lagoons, shallow waters and marshes. They have been determined in these environments in concentrations varying from 10–20 ng/l up to 127 μg/l.[6–9]

Different aquatic organisms such as clams, crayfish, rainbow trout and salmon have been analyzed and concentrations varying from a few ng/g up to 666 μg/g after 8 days of application, with mean values of 20–100 ng/g, have been reported.[10–13]

Information on point source inputs of the OP compounds into the Mediterranean is very limited. Most of the information available is related to levels in surface waters in Italy. In the river Tiber maximum concentrations of malathion of 0.5–0.6 μg/l have been detected during 1970–73.[14] Mean values of methyl-parathion and ethyl-parathion were below 0.1 μg/l, except in 1976, where a concentration of 4.4 μg/l was recorded.[15] During the period 1982–84 the OP levels were below the detection limits.[16] In Greece, the level of diazinon at the river Kalamas had a maximum value of 0.053 μg/l in summer and below 0.001 μg/l in winter.[17] Fenitrothion has been detected in Valencia (Spain) with levels from 0.05 to 2.02 μg/l, during the period 1983–85.[18] In France, ethyl-parathion and methyl-parathion have been detected in river waters of the Rousillon and the Saone river at levels of 0.01 μg/l.[19,20] In the Camargue region values were below 8 μg/l.[21] In Italy, during 1985 and 1987, tri-iso-butyl phosphate (TiBP), tri-butyl phosphate (TBP) and tris-2-chlorethyl phosphate (TCEP) have been monitored in the rivers Po and Lambro. Levels varied from non detectable (below 0.01 μg/l) up to 0.5 μg/l.[22,23] In Spain, in the rivers Llobregat and Besos, concentrations of TiBP and TBP during 1985–86 were below 1 μg/l with incidental values of 14–24 μg/l.[24]

In this paper, the concentrations of OP compounds in waters and biota from two Western Mediterranean rivers (e.g. the Llobregat and Ebro) and adjacent waters are reported for the first time (Figure 1). Samples of mussel (*Mytilus galloprovincialis*) and red mullet (*Mullus barbatus*) were collected at stations C and D, and samples of mosquito fish (*Gambusia affinis*) at station E, located at the channels of the Ebro delta.

To this end, analytical methodologies have been improved using a clean up procedure with silica gel and gel permeation chromatography (GPC) for water and biota samples, respectively.[25–29] As regards the identification of OP pesticides, the selectivity and sensitivity of capillary gas chromatography-negative chemical ionization (NCI) MS has been emphasized.[30,31] For non-pesticide OP compounds, such as TBP and TCEP, positive chemical ionization (PCI)-MS has been recommended.[32,33]

EXPERIMENTAL SECTION

Materials

The solvents ethyl acetate, cyclohexane and isooctane were of pesticide grade

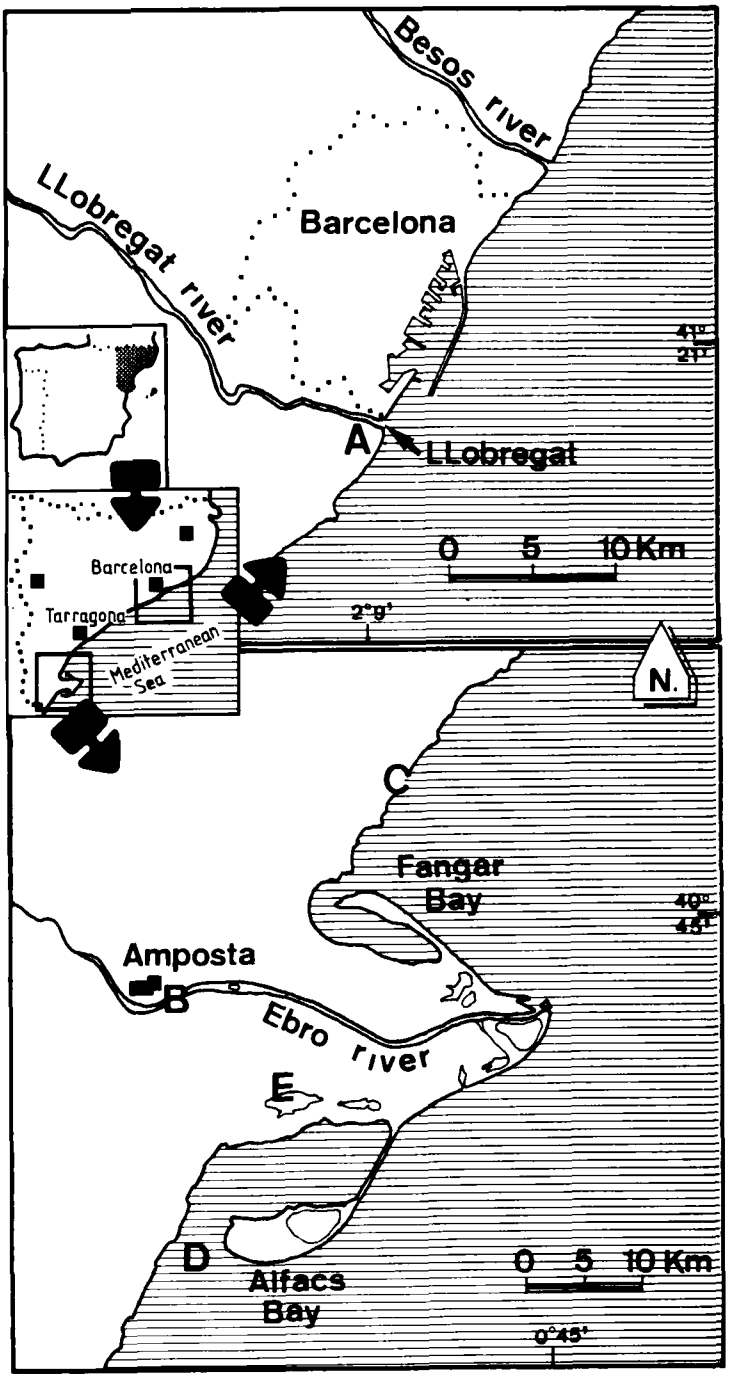

Figure 1 Map of the Llobregat and Ebro estuaries with their monitoring stations.

(SDS, Peypin, France). Organophosphorus pesticide standards were obtained from Polyscience (Niles, IL, USA) except ethyl-paraoxon and methyl-paraoxon that were purchased from Dr. Su. I. Ehrenstorfer (Augsburg, FRG). TCEP was a gift from S. Galassi (Irsa-CNR, Brugherio, Italy) and TiBP and TBP were obtained from Kodak (Rochester, NY USA).

Sample Preparation

Water: sample of 1 to 4 l were extracted 2–3 times with 30 ml of methylene chloride during 5 min. The combined extracts were dried over anhydrous sodium sulphate and concentrated in a rotary evaporator to near dryness. The concentrate was redissolved in 0.5–1 ml of ethyl acetate and submitted to a clean up using 5 g of silica gel prior to GC analysis. Recoveries varied from 92 to 100%.[25]

Fish: an homogenate of fish tissue (1–2 g) was mixed with 20–30 g of anhydrous sodium sulphate and extracted for 18 h with ethyl acetate in a soxhlet apparatus. The solvent extract (100 ml) was evaporated just to dryness and the residue was dissolved in 50–100 μl of ethyl acetate-cyclohexane (1:1) for further fractionation by gel permeation chromatography (GPC).

Chromatographic Analysis

GPC: eluent delivery was provided by a 64 high-pressure pump (Knauer, Homburg, FRG); a Vari-chrom UV-VIS (Varian, Sunnyvale, CA, USA) was used as detector at 254 nm. Samples were injected via 50 and 150 μl loops from Rheodyne (Cotati, CA, USA). Stainless-steel columns (450 × 10 mm i.d.) (Tracer Analítica, Barcelona, Spain) packed with Bio-Beads SX-3 (mesh size 200–400) (Bio-Rad Labs., Richmond, USA) were used.

The GPC eluent was ethyl acetate-cyclohexane (1:1) pumped at 1 ml/min. The collection time was from 22 to 34 min and the mean recovery varies from 64 to 85% with a relative standard deviation of 10–12% (n = 10).

GPC-NPD: GPC fractions were evaporated just to dryness, dissolved in iso-octane and injected onto the GC 6000 Vega series (Carlo Erba, Milan, Italy) equipped with a nitrogen-phosphorus detector (NPD). A 30 m × 0.25 mm i.d. coated with 0.12 μm of chemically immobilized SPB-5 (Supelco, Bellefonte, PA, USA) was used. Hydrogen was the carrier gas at 50 cm/s and helium the make up gas at 30 ml/min. The injector and detector temperatures were held at 300 and 320 °C, respectively. The column was programmed from 60 to 300 °C at 6 °C/min, keeping the final temperature for 15 min.

GC-MS: a HP 5988A instrument interfaced to a HP 9825A data system (Hewlett-Packard, Palo Alto, CA, USA) was used. Helium was the carrier gas at 30 cm/s. The same column as for GC-NPD analyses was used, programmed from 60 to 90 °C at 15 °C/min and from 90 to 300 °C at 4 °C/min, keeping the final temperature for 10 min. Methane was used as the reagent gas at 1.5 Torr. The transfer line, ion source and analyzer were held at 280, 200 and 230 °C,

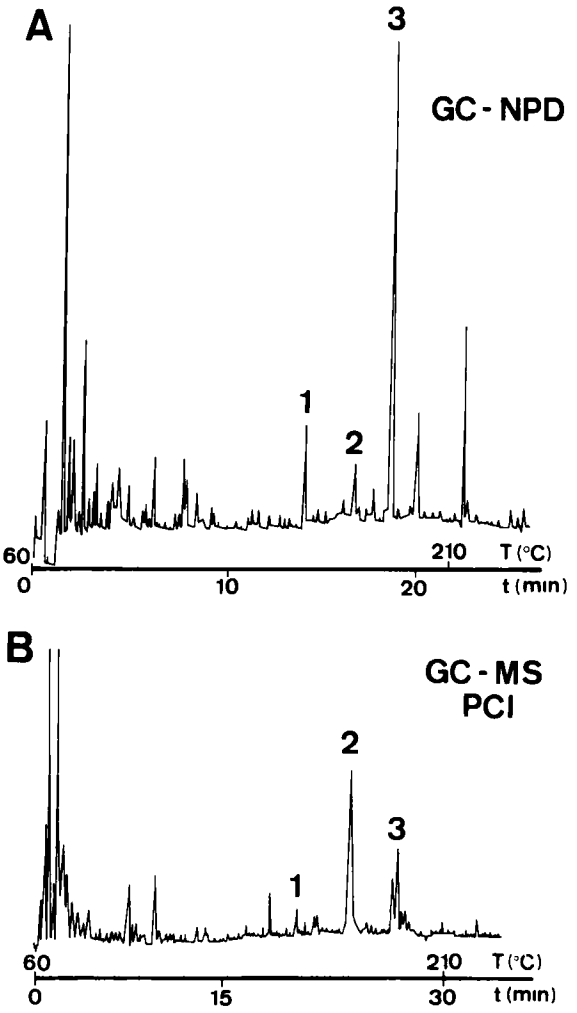

Figure 2 (A) Capillary-GC-NPD trace of a Llobregat river water extract; (B) Capillary-GC-PCI-MS trace of the same extract. The identified peaks are (1) TiBP (2) TBP and (3) TCEP.

respectively. Scan acquisition was from m/z 40 up to 550 at 1.68 scan/s. Positive and negative chemical ionization (PCI and NCI, respectively) modes of operation were used for confirmatory purposes of the different OP compounds.

RESULTS AND DISCUSSION

Identification of Organophosphorus Compounds

Water samples: a representative capillary-GC-NPD profile of a water extract from the Llobregat river is shown in Figure 2A. Compounds identified correspond to TiBP (No. 1), TBP (No. 2) and TCEP (No. 3). In Figure 2B the capillary GC-

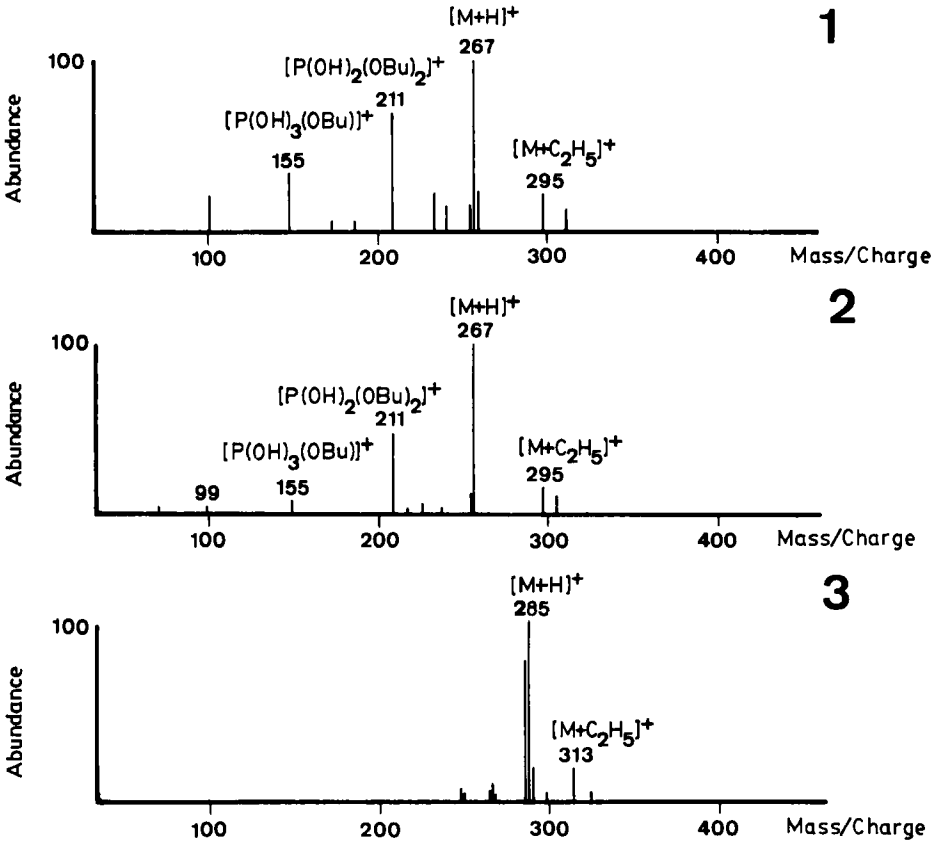

Figure 3 PCI-mass spectra of the organophosphorus compounds identified in Figure 2.

PCI-MS trace of the same extract is shown. Compounds 1–3 have been identified by PCI-MS, and the corresponding mass spectra are shown in Figure 3.

It is interesting to note that the mass spectra of TiBP (No. 1) and TBP (No. 2) are virtually identical, exhibiting a $[M+H]^+$ as base peak at m/z=267. Diagnostic ions at m/z=155 and 211 are characteristic of this group of compounds, corresponding to $[P(OH)_3(OBu)]^+$ and $[P(OH)_3(OBu)_2]^+$, respectively.[33] It is also interesting to mention the formation of the fragment at m/z=295, corresponding to $[M+C_2H_5]^+$, due to the reagent ions generated by the methane. In contrast, when the EI-MS mode is used, less sensitivity but more structural information is obtained in the mass spectra which allows one to differentiate between TiBP, with fragment ions at m/z=139 and m/z=195, and TBP, with a fragment ion at m/z=125.[24,33] Compound No. 3, identified as TCEP, exhibits a base peak at m/z=285 and a second fragment at m/z=313, corresponding with $[M+H]^+$ and $[M+C_2H_5]^+$, respectively.[32] These compounds are currently used as plasticisers and industrial solvents, and have been identified by Galassi *et al.* in rivers of Northern Italy.[22,23]

Biota samples: Results for a biota extract of *Gambusia affinis* are given in Figure 4. The GC-NPD chromatogram is shown in Figure 4A. In Figure 4B the

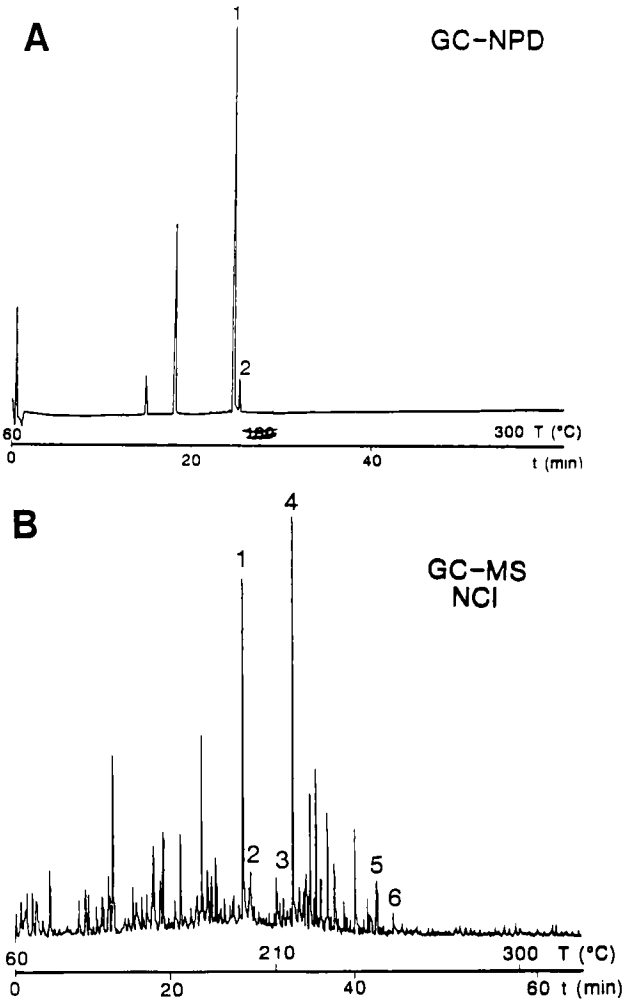

Figure 4. (A) Capillary-GC-NPD trace of a *Gambusia affinis* extract; (B) Capillary-GC-NCI-MS of the same extract. The identified peaks are (1) fenitrothion, (2) malathion, (3) tetrachlorvinphos, (4) p,p'-DDE, (5) azinphos-ethyl and (6) coumaphos.

corresponding NCI-MS chromatogram is shown. the NCI-MS chromatogram is seen to be less selective than the NPD one, which is to be expected because of the fact that most compounds exhibiting electron capture ability will also respond under NCI-MS conditions. OP pesticides identified in the chromatogram of Figure 4B were peaks 1,2,3,5 and 6. Peak No. 4 is p,p'-DDE which is detected in the same extract because it coelutes with the organophosphorus pesticides under the GPC conditions used in this work.[34] As regards the sensitivity the NCI-MS chromatogram is slightly better than the NPD, thus allowing the detection of compounds 3,5 and 6.

The NCI mass spectra of compounds 1–6 are shown in Figure 5. Malathion (peak No. 2) and tetrachlorvinphos (peak No. 3) exhibit as base peak their corresponding specific group fragment, which has a value of 50% for azinphos-

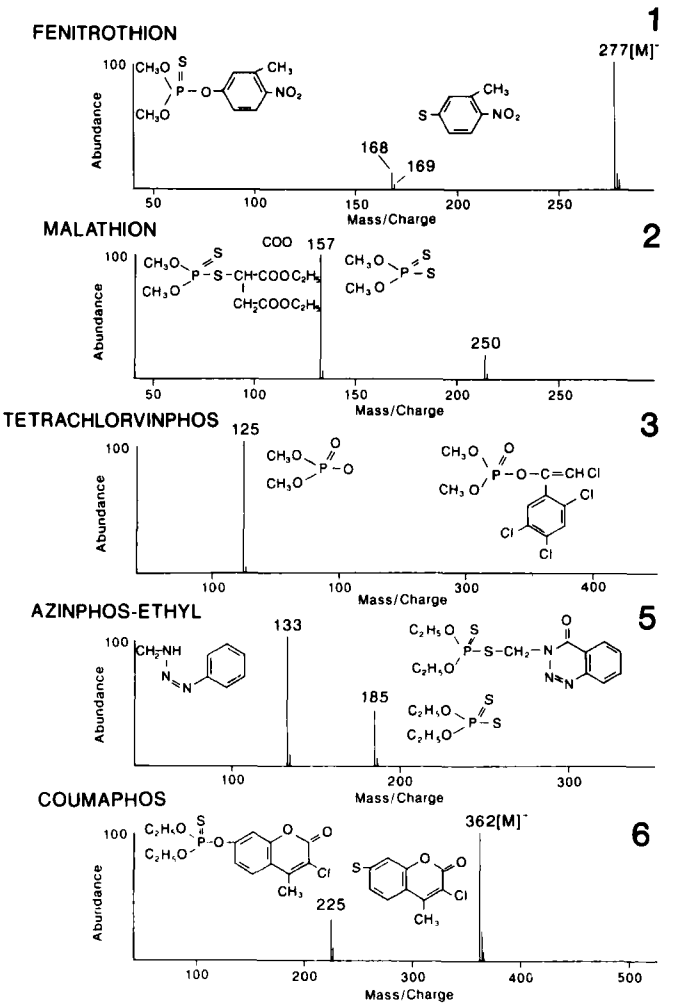

Figure 5 NCI-mass spectra of pesticides identified in Figure 4.

ethyl (peak No. 5). Fenitrothion and coumaphos show the $[M]^-$ ion as the base peak owing to the fact that the Z moiety contains an aromatic ring, which stabilizes the $[M]^-$ ion by electron delocalization.[31] The formation of the fragment ions at m/z 168 and 225 for fenitrothion and coumaphos, respectively, is a typical feature of phosphorothionates where the Z substituent is a phenyl group. In these compounds the thiophenolate anion is favoured over the formation of a phenolate anion, so that phenyl transfer followed by cleavage of the P–S bond occurs preferentially.[30,31,35]

Quantitation of Organophosphorus Compounds

Water samples: the monitoring survey was carried out from March to June 1988. The concentrations are given in Table 1. The average values of 0.05–0.1 μg/l with

Table 1 Organophosphorus compounds in water samples of the Llobregat river (Station A) and the Ebro river mouth (Station B)[a]

Compound	*Station A*				*Station B*	
	March 88	*April 88*	*May 88*	*June 88*	*March 88*	*April 88*
TiBP	n.d.	n.d.	0.01	0.9	n.d.	n.d.
TBP	0.3	0.05	0.001	0.05	n.d.	0.02
TCEP	n.d.	n.d.	0.4	0.3	n.d.	n.d.
Diazinon	n.d.	n.d.	n.d.	0.03	n.d.	n.d.
Parathion-ME	0.03	n.d.	n.d.	n.d.	n.d.	n.d.

[a]Concentrations expressed in μg/l.
n.d. = not detected (below 1 ng/l)

Table 2 Organophosphorus pesticides in biota samples of the Ebro Delta, Fangar Bay (Station C) and Alfacs Bay (Station D)[a]

Compound	*Station C*		*Station D*		
	April 88 M. barbat	*May 88 M. gallop*	*April 88 M. barbat*	*May 88 M. gallop*	*July 88 M. barbat*
Diazinon	1.2	n.d.	n.d.	n.d.	n.d.
Parathion-ME	2.2	n.d.	n.d.	n.d.	0.5
Paraoxon-ET	16	n.d.	n.d.	n.d.	n.d.
Fenitrothion	16	n.d.	n.d.	n.d.	n.d.

[a]Concentrations expressed in ng/g fresh weight.
n.d. = not detected (below 1 ng/g)

Table 3 Organophosphorus pesticides in samples of *Gambusia Affinis* from the Ebro Delta (Station E)[a]

Compounds	*April 87*	*May 87*	*July 87*	*Sept. 87*	*Nov. 87*	*Feb. 88*
Dioxathion	n.d.[b]	n.d.	28	11	8	22
Ronnel	2	1	n.d.	2	1	9
Fenitrothion	n.d.	1.	5	27	187	306
Malathion	n.d.	n.d.	n.d.	1	2	17
Fenthion	n.d.	n.d.	n.d.	n.d.	7	35
Tetrachlorv.	n.d.	n.d.	n.d.	9	1	18
Azinphos-E	n.d.	n.d.	n.d.	18	n.d.	31
Coumaphos	n.d.	n.d.	n.d.	12	n.d.	16

[a]Concentrations expressed in ng/g fresh weight.
[b]n.d. = not detected (below 1 ng/g).

incidental values of 0.9 μg/l for non pesticide OP compounds are similar to those reported by Galassi *et al.*[22,23] for the mouth of the river Po. They are indicative of the contamination produced by industries located along the river course. The highest level was found at Station A, at the Llobregat river, where intense industrial activities are carried out. For the first time TCEP has been unequivocally determined in Spanish rivers with levels up to 0.3 μg/l, reflecting its use in the monitored area. As regards OP pesticides, their concentrations are rather low, from non detectable (below 1 ng/l) to 0.03 μg/l.

Biota samples: the concentrations of OP pesticides in biota samples are given in Tables 2 and 3. The monitoring survey in Stations C and D has been undertaken

from April to July 1988, and Station E from April 1987 to February 1988. Non pesticide OP compounds have not been found in the samples, probably due to their higher solubility and/or volatilization.[4]

As regards the levels indicated in Table 2 and 3, it should be mentioned that the OP pesticide concentrations were at the low ng/g level. The highest concentration was found for fenitrothion, which currently is the main rice borer OP insecticide aerially applied by aircraft in Station E. Maximum levels are found in February, probably due to the fact that this is the dry period of the year, so that the water, from which the mosquito fish was sampled, contains higher concentrations of pesticides.

CONCLUSIONS

The concentrations of OP compounds monitored in two different estuaries of the Western Mediterranean, the Llobregat and Ebro rivers, (Spain) are consistent with their solubilities. The phosphates, which are not used as pesticides, have a higher water solubility than the OP pesticides; for example, those of TBP and TCEP are 280 mg/l and 7000 mg/l, respectively, as against 24 mg/l for ethyl-parathion. Consequently, the former compounds are found at higher concentrations in river water (eg. the Llobregat river) affected by industrial activities. On the other hand, only OP pesticides – and not OP compounds of industrial source – were found in biota samples. This can be related to the higher bioconcentration factors (BCF) of OP pesticides. While TBP and TCEP exhibit BCF for different fish species of 1–30, the BCF for OP pesticides vary from 70 up to 550, with a value of 180 for fenthion.[4,36]

Acknowledgements

This work has been supported by the Food and Agriculture Organization and the United Nations Environment Programme (FAO-UNEP).

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